1 Anti-hemorrhoid Evaluation of Selected Medicinal plants used in

2 North-East Nigeria for the Treatment of Hemorrhoids (Pile)

3 ABSTRACT

4 A medicinal plant is any plant in which one or more of it organs contain substance that can be used for therapeutic purpose or which are precursors for the synthesis of useful drugs. In 5 6 this research, four(4) selected plants were screened for antihemorrhoid activities in mice. 7 The extracts were obtained from whole plant or part of plants such as root, stem, leaves and 8 seeds and include the following plants: Khaya senegalensis, Euphorbia hirta, Parkia 9 biglobosa, Newbouldia leavis and Prosopis africana. Hemorrhoid (pile) was induced in 10 group of five mice of five animals per group using Jatropha oil(Jatropha curcas, 11 Euphorbiaceae)(I.P) and using Pilex granule as the control drug. Group I received 5mg/kg12 Pilex granule, and 200, 250, 300, 350 mg/kg b.w of A. leiocarpus, N. leavis, P. africana, and 13 KEP for groups II, III, IV and V respectively. A. leiocarpus and KEP(mixture of K. senegalensis, E. hirta and P. africana) showed the best antihemorrhoidal activities in mice 14 than the other plants and compared with the standard drug Pilex granule. However, all the 15 plants extracts showed significant rectoanal coefficient at potent levels. The study showed 16 17 that the extracts of the plants investigated possessed antihemorrhoid activities with A. 18 leiocarpus demonstrating the **best** activity in mice.

19 Keywords: Anti-hemorrhoid, medicinal plants, mice, pilex granule, Jatropha oil.

20 Introduction

Hemorrhoids represent the dilation of varicose of the vessel of the superior of inferior rectal 21 plexuses of veins. They have be noted common human affliction from the down history. The 22 23 exact incident in population of developing countries has not been determined but in spite of 24 ascertain to the contrary. The condition is frequently encountered in developing country [1]. 25 Various dilation of the internal hemorrhoids; physiological dilation present already in infancy is presumed to develop into varicosities under the influence of wide range of factors. The 26 predisposing factors include hereditary, age, sex pregnancy are the prepared state and even 27 paramount. The precipitating factors comprise cathartic abuse, diarrhea, enemata, 28

29 constipation infection and spasms or a tony of the oral sphincter obesity and arise in extra-30 abdominal pressure [2]. There are two types of piles, internal piles and external piles. Internal 31 piles expand inside, along the anal. The common symptoms of internal piles are the painless 32 blood loss. The internal piles are the totally prolapsed. External piles extend close to the 33 anus. The colour of external piles is same as the skin. The outside piles forms thrombus. The 34 outside piles are painful. When the external pile ruptures it bleeds. The blood loss is more 35 disturbing and blood loss is typical cause for considering a doctor. Prolapsed is on the other 36 hand, oral dysfunctional special effect, and the other undeniable warning sign soreness, 37 impatient are fewer dependable problem solving criterion [3].

38 MATERIALS AND METHODS

39 Plant Collection and Identification

40 The plants species (Parkia biglobosa, Prosopis africana, Euphorbia hirta, Khaya 41 senegalensis, Newbouldia leavis and Anogeissus leiocarpus) were collected from Bali town 42 and were identified by Mr. Cletus A. Ukwubile of Science Laboratory Technology 43 Department, Federal Polytechnic Bali, where voucher numbers were deposited for the plants 44 Attempt has been made to expel scyballous masses from the rectal by traditional 45 medical practitioners using different plant species in either of the following preparation 46 methods: concoction, decoction and maceration. This research aims to identify the plant 47 species used for the treatment of such ailment and also to identify the plant species which has the best anti-hemorrhoids properties, also the ecology, scientific names and method of 48

50 **Preparation and Extraction of Plant Materials**

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51 The plants were air-dried for two weeks and then, plants parts were ground into powder and 52 weighed and stored for onward use. One of the plants was extracted with aqueous solution

preparation of the drug and also to make a herbarium press of the plant species.

53 (*Newbouldia leavis*) while others were extracted with absolute ethanol 99.1% (v/v), which
54 was soaked for 24 h using cold maceration technique.

55 Grouping of Swiss albino rats

56 The animals were grouped into five (5) groups of 5 animals according to each plant extracts.

57 Experimental Animals

Inbred male and female Swiss albino mice (18-29g weights) that were housed in standard 58 conditions of temperature $(22 \pm 3^{\circ}C)$, relative humidity $(55 \pm 5\%)$, and light (12h light-dark 59 cycle) before and during the study were included in the experiment. They were fed with 60 61 standard pellet diet (obtained from animal house of Department of Pharmacology and 62 Clinical Therapeutics, Ahmadu Bello University Zaria) and water ad libitum. All the 63 experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC) 64 of the Ahmadu Bello University Zaria and Health Research Extension Act of 1985(Public 65 Law November 20, page 99-158) USA. The animals received humane care as per the guidelines prescribed by committees for the purpose of control and supervision of control 66 67 and supervision of experiments on animals (CPCSEA), the Ministry of Environment and 68 Forests, Nigeria.

69 Experimental Protocols

Two sets of experiments were carried out. The first set was used to improve an existing experimental model of hemorrhoids mentioned by [3], and to validate the same by using pilex granules (PG), *Newbouldia leavis* extract (NE), and a combination of both extracts. The protocol was designed to quantify the extent of plasma exudation and to determine the levels of inflammatory cytokines such as TNF- α and IL-6 associated with hemorrhoids. In the second set, the effect of PG, AL, and a combination of some plant extracts were further

76 confirmed by determining the rectoanal coefficient (RAC), severity score, and the77 histopathological evaluation[4].

Evaluation of Anti-hemorrhoid property against Jatropha oil-induced hemorrhoid in mice

80 Mice of both sexes (20-29g) were randomized based on their body weights and were divided into 5 groups (G-1-G-5), with each group consisting of 5 animals (n=5). G-5 animals 81 82 received PG (Pilex granule) (10mg/kg) and served as positive control; G-1 animals received 83 AL (200mg/kg):G-2 and G-3 animals received NL and KEP (200 and 400mg/kg b.w; i.p., 84 respectively). Haemorrhoids were induced to all the groups, except normal control group, by 85 applying croton oil preparation. 24h hours after induction, all the animals were subjected to 86 respective treatment as assigned to the groups once daily for five days. On the fifth day, 1 h 87 after the treatment, all the animals were euthanized by exsanguinations under deep isoflurane 88 anaesthesia and rectoanal tissues (20mm in length) were isolated. They were evaluated for the 89 severity score, weighed, and fixed in 10% formalin solution for histological examination.

- 90 The RAC was calculated using the formula
- 91 *Rectoanal coefficient* = <u>Weight of rectoanal tissue (mg</u>
- 92

Body weight (g)

Histological observation of the rectoanal tissue was carried to determine the appearance
of inflammatory cells, congestion, haemorrhage, vasodilatation, and medium to high degrees
of necrosis [5].

96

97 **RESULT**

98 Table 1: LD₅₀ Determination of Jatropha oil from *Jatropha curcas*

Dosage (mg/kg)	No of animal died _/ no of animal survive

100	0/5
200	0/5
250*	1/4
300	ND

99 $LD_{50} = 1118 \text{ mg/kg b.w}$ (Lorke , 1983), ND (not determine), * toxic dose

100 Table 2: Effect of Jatropha oil on the body weights of mice before and after induction

Test groups	Weight Before (g)	Weight After (g)
Group I Control 100mg/kg	29.6	25.2
Group II 200mg/kg	28.6	24.1
Group III 250mg/kg	25.2	20.1
Group IV 300mg/kg	28.0	22.2
Group V 350 mg/kg	26.6	20.2

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102 Table 3: Effect of extracts on rectum after drug administration (i.p)

Extract dose (mg/kg b.w) (n=5)	Rectoanal coefficients (g)	Inference
Group I Control Pilex (5mg)	0.6 ± 0.22	Moderate healing
Group II AL 100	$0.1 \pm 0.20^{\text{TM}}$	*Strong healing
Group III NL 200	0.4 ± 0.18	Moderate healing
Group IV PA 400	0.3 ± 0.15	Moderate healing
Group V KEP 600	$0.2 \pm 0.10^{\text{TM}}$	*Strong healing

103 AL (Anogeissus leiocarpus), NL (Newbouldia leavis), PA (Prosopis africana), KEP (Khaya senegalensis,

104 Euphorbia hirta, Parkia biglobosa), Results are means ± SEM. The lower the values, the more efficacy the

105 drug, TM More efficacy.



Figure 1: Medicinal plants effects on rectoanal tissue in mice in Jatropha oil-induced hemorrhoids; a;
hemorrhoid induced in recto anal tissue, b-c; healing of tissue after drug administration with *Anogeissus leiocarpus*, d; mouse developed pile after five days of induction with Jatropha oil.

113 DISCUSSION

114 Discussion

115 It is well proved that hemorrhoids are a pathological condition, which is characterized by a 116 severe vasodilation at the rectoanal region, which leads to inflammation of the surrounding 117 tissues, thus further leading to secondary complications such as extravasations of fluid into 118 interstitial space mainly due to increased vascular permeability and migration of large 119 quantity of inflammatory cells (granulocytes and monocytes)[6].

In the present study, Jatropha oil from seeds of *Jatropha curcas (Euphorbiaceae)* has been used as inducer/phlogiston agent to induce experimental hemorrhoids. Jatropha oil causes inflammation due to the release of soluble factors involving inflammatory lipid metabolites [7].These factors, alone and/or in combination, regulate the activation of resident cells (Fibroblasts, endothelial cells, macrophages, and mast cells) and newly recruited inflammatory cells (Monocytes, lymphocytes, neutrophils, and eosinophils) leading to systemic response to inflammation [8-9].

127 The normal control group showed normal cell architecture of the rectoanal region. The 128 results showed the loss of weights in the animals after induction (Table 2), which is a 129 symptoms of the disease. However, intraperitoneal administration of plant extracts of AL, 130 NL, PA, and KEP showed remarkable vasoconstriction of the rectum (Table 3). The greatest 131 healing of the rectum were shown by AL (Anogeissus leiocarpus) and KEP (K. senegalensis, 132 *Euphorbia hirta, Parkia biglobosa*), and these signify the constriction of the mucosa linings 133 of the anus in the mice by the plant extracts. These results were comparable with that of the 134 standard control drug (Pilex). All the extracts produced a better rectoanal coefficient values 135 than the first line drug (Table 3).

136 Conclusion

Medicinal plants are a source of many biological ingredient which cannot be ignored. The study therefore showed that extracts of *Anogeissus leiocarpus, Khaya senegalensis, Euphorbia hirta, Parkia biglobosa* and *Newbouldia leavis* posses antihemorrhoid properties in mice, and can be use as medication for the treatment of hemorrhoid (pile). These plants thus, represent sure source towards the development of orthodox medicine for the treatment of piles than surgery, which normal is expensive and risky. However, the precise molecular

143 mechanism behind the antihemorrhoidal activities of these plant extracts need to be explored

in future studies.

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